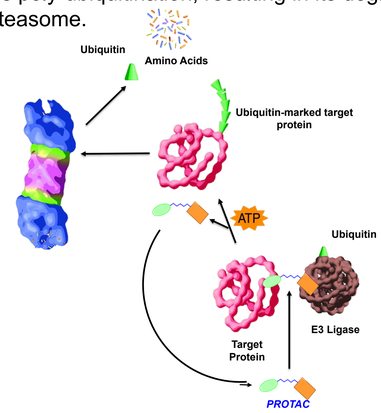
The Ohio State University College of Pharmacy, Division of Medicinal Chemistry and Pharmacognosy

Development of Proteolysis-Targeting Chimeras as a Target for Hepatocellular Carcinoma

Galina Rozenberg; Bo Tokarski, PhD; Christopher Coss, PhD; James Fuchs, PhD

INTRODUCTION

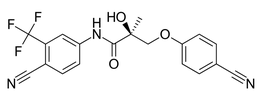
Proteasomes are protein complexes which can be targeted for protein degradation via proteolysis. Molecules known as proteolysis-targeting chimeras, or PROTACs, can be designed to target a specific protein of interest, as well as an E3 ligase protein which causes the target protein's poly-ubiquitination, resulting in its degradation by the proteasome.



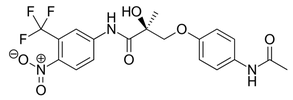
BACKGROUND

Hepatocellular carcinoma is one of the most common types of liver cancer and is the third leading cause of cancer death worldwide. Androgen receptors have been studied as potential targets for the treatment of hepatocellular carcinoma. Androgen receptors ligands can be incorporated into PROTACs to be used in the targeted degradation of the androgen receptor.

Selective Androgen Receptor Modulators

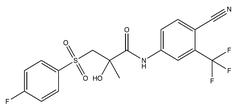


Enobosarm

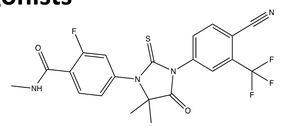


Andarine

Antagonists



Bicalutamide



Enzalutamide

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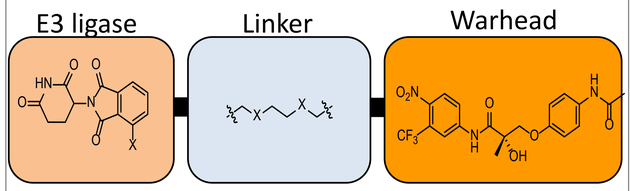
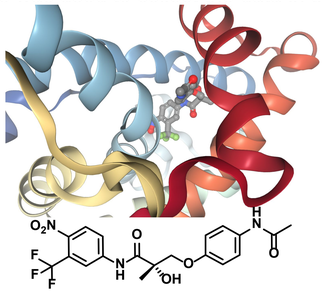
PROTACs are made up of three regions: the warhead, the linker, and the E3 ligase ligand. The warhead is designed to bind to the protein of interest, in this case the androgen receptor. The E3 ligase ligand is designed to bind to the E3 ligase protein. The E3 ligase recruits the E2 conjugating enzyme which poly-ubiquitinates the target protein, in this case the androgen receptor, and flags it for degradation. The linker region is the region which connects the hydrophobic tag to the warhead region and can be adjusted to alter the biological activity of the molecule.



Based on work previously done in the Fuchs lab for the design of protein degraders against leukemia, thalidomide analogues were selected as the E3 ligase. This decision is based on the relative ease of thalidomide synthesis as compared to VHL ligands. The linker would be selected from alkyl and polyethylene glycol units as suggested by Maple et al. Andarine was selected as the warhead to target the androgen receptor. The relative connectivity of these pieces remained to be determined, particularly the point of attachment for andarine.

METHODS

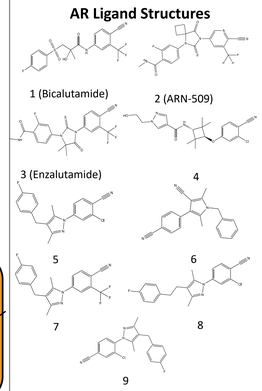
The Protein Data Bank was used to study the x-ray crystal structures of androgen receptor modulators to determine binding mode and identify a suitable point of attachment for the linker. As an example, andarine is shown below. In this crystal structure, the amide group on the right side of andarine is shown to be projecting out of the binding pocket. Therefore, linker attachment should be at this site. A database consisting of 1662 PROTACs and 202 warheads was used to collect the data in the results.

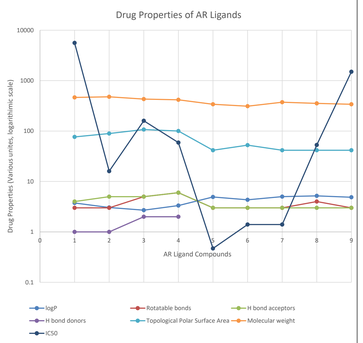


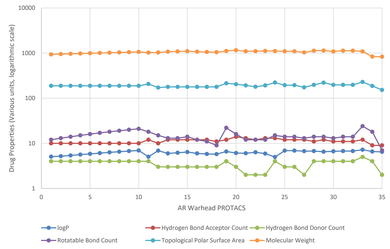
Andarine (S-4)

RESULTS

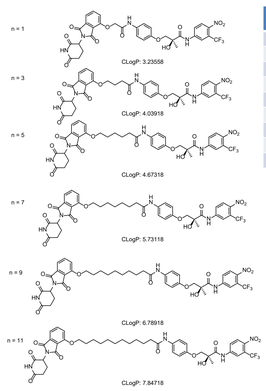
AR Ligand Structures







	Max (AR)	Min (AR)	Max (AR PROTAC)	Min (AR PROTAC)
Molecular Weight	477.443	311.388	1154.355	829.398
logP	5.19	2.7	7.22	4.97
Hydrogen Bond Acceptor Count	6	3	14	9
Hydrogen Bond Donor Count	2	0	5	2
Rotatable Bonds	6	3	24	7
Topological Surface Area	107.26	41.61	228.88	152.15
IC50 (9)	5600	0.47	N/A	N/A



	# Atoms in linker	MW	ClogP	tPSA
1	1	713.58	3.23	232.25
2	3	741.63	4.03	232.25
3	5	769.69	4.67	232.25
4	7	797.74	5.73	232.25
5	9	825.80	6.79	232.25
6	11	853.85	7.85	232.25
7	5 (1 - O)	771.66	3.70	241.48
8	8 (2 – O)	815.71	3.53	250.71
9	11 (3 – O)	859.77	3.35	259.94

CONCLUSIONS

Most linkers are designed using simple alkyl or polyethyleneglycol chains. An analysis of the drug properties of andarine-based PROTACS shows that the alkyl linkers between 2 and 7 atoms in length have ClogP values in the 4-6 range, which according Maple et al. is expected to give good activity due to permeability. The shortest chain (n = 1) gives a ClogP of 3.23, but more importantly may not be long enough to allow formation of the ternary complex, the PROTAC molecule forming a bridge between the two proteins (AR and E3 ligase). The longest chains examined (n = 9 and n = 11) have ClogP values that are above 6, suggesting that they may be too lipophilic. The PEG linked analogues tell a completely different story. In this case, ClogP decreases as a function of chain length, suggesting that lengthening of the chain may be counterproductive from the perspective of potency. In addition, longer chain lengths of the PEG series are associated with increased polar surface areas, while the tPSA values for the alkyl chains remain constant.

FUTURE DIRECTIONS

PROTACs could be a potential treatment for not only hepatocellular carcinoma, but also other types of cancers as well. Based on the research of AR ligands and PROTACS, synthesis of the most promising molecules will be synthesized and studied in the future.

ACKNOWLEDGEMENTS

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